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Genetics

Restriction fragment length polymorphism of the human c-*fms* gene

(oncogene/homozygote/heterozygote)

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ABSTRACT **By using blot hybridization with a v-*fms* probe, a polymorphism for *Eco*RI, *Hin*dIII, and *Bam*HI restriction endonuclease sites associated with the human c-*fms* locus was observed in a random adult population. This restriction fragment length polymorphism can be explained on the basis of the existence of two alleles, *a* and *b*, and is due to a short (≈500 base pairs) deletion characteristic of allele *a*. The distribution in the analyzed population (48 unrelated individuals) is 23% heterozygotes *ab*, 75% homozygotes *bb*, and 2% homozygotes *aa*. Though the inheritance of this polymorphism follows a Mendelian pattern, the children from couples *ab* × *bb* are of the following genotype: 74% *ab* and 26% *bb*. These deviations from the expected frequencies of 50% suggest a selective pressure in favor of heterozygotes.**

Cytogenetic studies first focused attention on the possible involvement of cellular oncogenes in malignant disorders (1–3). Studies on chromosomal aberration were then followed by biochemical approaches that, in some cases, confirmed that chromosomal translocations might directly affect the oncogenes (4–10). Such results stimulated a systematic search for rearrangement of oncogenic loci, for which a role in pathological situations and chromosomal aberrations could be foreseen. However, before deciding whether a particular restriction enzyme pattern for one specific locus has been modified as a consequence of a chromosomal rearrangement or whether a particular restriction pattern could designate a population at risk, one must know the original pattern and also the details of any restriction fragment length polymorphism (RFLP) (11) that may exist for that particular locus.

Oncogenes have been rather well conserved during evolution, hence polymorphism is probably not frequent among them. In this context, detection of a polymorphism for a specific oncogene might have some consequences when the panel of DNA samples studied is well known for many other genetic markers.

In this study, we describe a RFLP for the c-*fms* locus detected with the restriction enzymes *Eco*RI, *Hin*dIII, and *Bam*HI. The human c-*fms* locus spans a distance of over 35 kilobases (kb) (12, 13) and, when probed with v-*fms* DNA, gives 3–7 fragments, depending on the enzyme used and the phenotype. Interestingly, this RFLP, which is due to the disappearance of a specific restriction site or a shift in mobility of a specific DNA band, is associated with a particular region of the c-*fms* locus; the other regions are without detectable polymorphism.

MATERIALS AND METHODS

DNA samples were provided by the Centre d'Etude du Polymorphisme Humain. Blood samples were obtained from Caucasian individuals born in France and belonging to families with more than four children. Formal consent was obtained from all families. Cellular DNA was extracted from buffy coat, by proteinase K and phenol/chloroform treatment (14, 15).

Serum and erythrocytes were tested for various genetic markers. Restriction enzymes (purchased from New England Biolabs or Boehringer Mannheim) were used to digest 10 μg of DNA according to specifications given by the manufacturers. The resulting fragments were fractionated by electrophoresis in 0.8% agarose gels (Sigma) and transferred by the method of Southern (16) to diazobenzyloxymethyl (DBM)-paper prepared as described (17, 18).

DNA probes were derived from a λ phage recombinant (19, 20). As shown in Fig. 1, an *Eco*RI–*Bam*HI subfragment (6.7 kb), containing the entire v-*fms* gene (3.1 kb) and some additional viral sequences, was subcloned in plasmid pUC9. From this recombinant plasmid, a subset of restriction fragments obtained by digestion with *Bgl* I and *Hin*d II was used to localize the region of polymorphism. DNA probes were labeled by nick-translation with [α-^{32}P]dCTP (>3000 Ci/mmol, NEN; 1 Ci = 37 GBq) to a specific activity >10^8 cpm/μg.

DBM-papers were prehybridized for 6–12 hr at 42°C in 50% (vol/vol) deionized formamide/5× NaCl/Cit (1× = 0.15 M NaCl/0.015 M sodium citrate)/50 mM sodium phosphate, pH 6.5/5× Denhardt's solution (1× = 0.02% bovine serum albumin/0.02% polyvinylpyrrolidone/0.02% Ficoll)/sonicated salmon sperm DNA (0.5 mg/ml).

Hybridizations were for 36–48 hr at 42°C in 50% deionized formamide/5× NaCl/Cit, 1× Denhardt's solution/20 mM sodium phosphate/1.5% dextran sulfate containing sonicated salmon sperm DNA at 0.1 mg/ml and radioactive probe at 10 ng/ml. After hybridization, the papers were washed with 2× NaCl/Cit/0.1% $NaDodSO_4$ at room temperature, followed by 0.1× NaCl/Cit/0.1% $NaDodSO_4$ at 50°C. Filters were autoradiographed on Kodak XAR-5 films with Cronex Hi-plus intensifying screens at −70°C for 2–6 days.

RESULTS

***Eco*RI Detection of a RFLP.** The *Eco*RI restriction patterns, obtained by hybridization with a complete v-*fms* probe, indicate three different phenotypes. In 36 out of 48 tested individuals (75%), the v-*fms* probe reveals four DNA fragments of 16, 13, 3, and 2.5 kb denoted b, c, d, and e, respectively. In 23% of the cases studied (11 out of 48 unrelated individuals), a band of 29 kb, denoted a, is observed in addition to the four others. In 1 case, a profile with only three bands (29, 3, and 2.5 kb) is observed. Thirty-eight children from nine couples chosen to represent different combinations of the various phenotypes were also studied. The results obtained are shown in Figs. 2 and 3. In every case, the fragment lengths have shown inheritance as Mendelian alleles through two generations.

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Abbreviations: RFLP, restriction fragment length polymorphism; kb, kilobase(s).

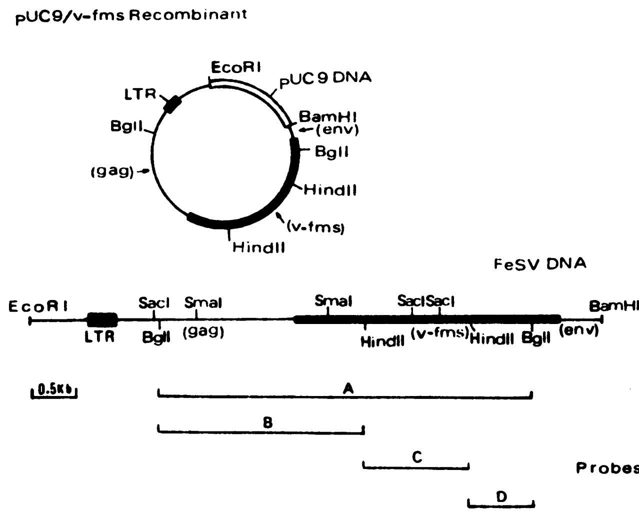
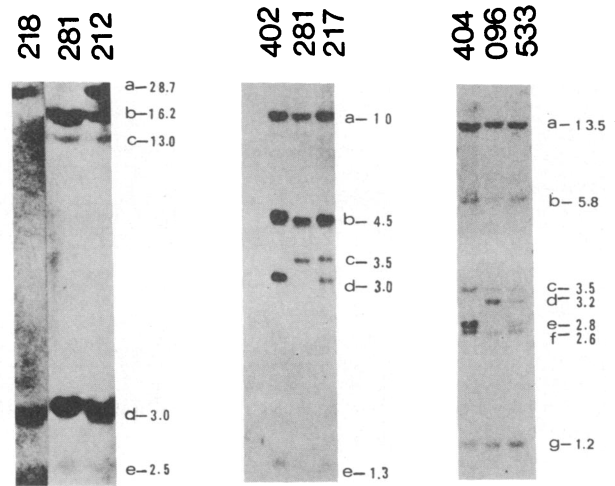


FIG. 1. Cloning of v-*fms* sequence in pUC9 and restriction map of a *Eco*RI–*Bam*HI fragment containing the entire v-*fms* gene. A, B, C, and D denote the various fragments used as probes to localize the region of polymorphism. LTR, long terminal repeat.

***Hind*III RFLP.** DNA samples from 24 individuals (also tested with *Eco*RI) were hydrolyzed with *Hind*III restriction endonuclease and probed with the complete v-*fms* DNA. As shown in Fig. 2, three different patterns were observed in this case, too. Pattern A is characterized by the presence of six bands of 13, 6, 3.5, 2.8, 2.6, and 1.2 kb (denoted a, b, c, e, f, and g). Pattern B is characterized by the disappearance of band e and its replacement by band d (3.2 kb). Pattern C shows the presence of both bands d and e.

Thirty-two children from eight couples were also analyzed. Results are summarized in Fig. 4.

***Bam*HI RFLP.** DNA samples from 10 individuals selected on the basis of results obtained with *Eco*RI and *Hind*III were hydrolyzed with *Bam*HI and hybridized with the complete v-*fms* probe. As shown in Fig. 2, three different patterns again were observed. Our interpretation of these data is that in the c-*fms* locus there are two alleles which give three genotypes with the distinct allelic combinations.



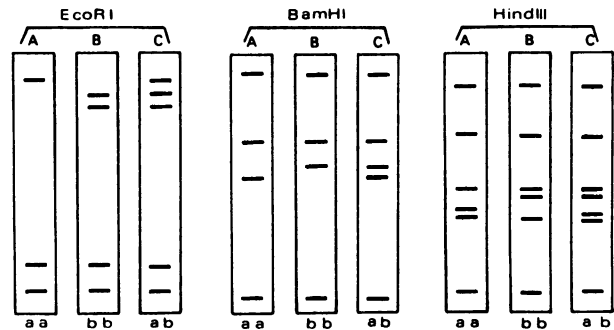
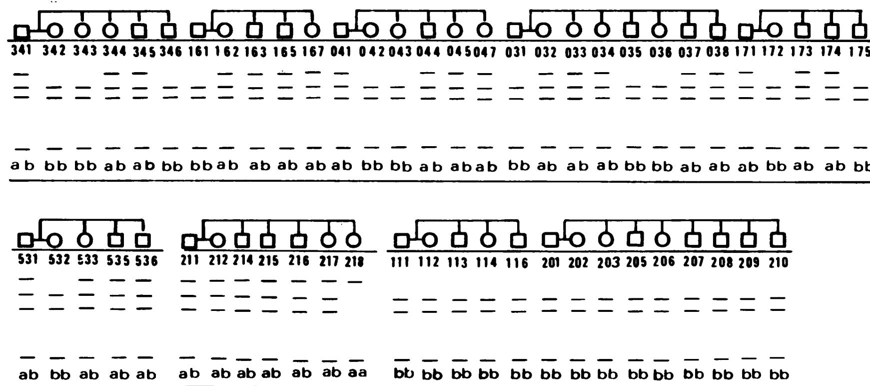


FIG. 2. (*Upper*) Autoradiograms of *Eco*RI, *Bam*HI, and *Hind*III digests of DNAs fractionated in 0.8% agarose gel, transferred onto diazobenzyloxymethyl (DBM)-paper, and hybridized with the nick-translated complete v-*fms* DNA probe (A in Fig. 1). The 2.5-kb *Eco*RI fragment e, though barely visible, was consistently observed in all DNAs analyzed. Sample identification numbers appear at the tops of the lanes. Fragments and their sizes (in kb) are given at the right of each autoradiogram. (*Lower*) A schematic representation of the patterns for the various phenotypes.

Localization of the Restriction Site Responsible for the *Eco*RI and *Hind*III RFLPs. Digestion of the v-*fms* recombinant by *Bgl* I generates a fragment of 4.3 kb corresponding to most of



	ab	bb
ab	4ab 1aa	17ab 6bb
bb	— —	10bb —

FIG. 3. Pedigree showing the genotype distribution of the *Eco*RI restriction fragments, as revealed with the whole-v-*fms* probe (A in Fig. 1), in different families. For each family, parents are represented on the left side.

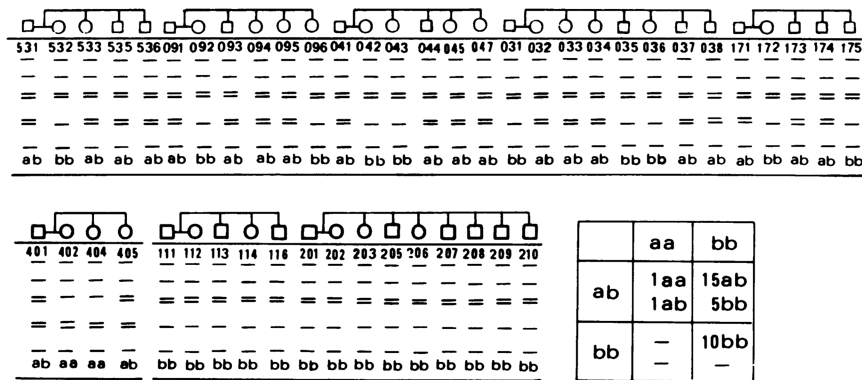


FIG. 4. Pedigree showing the genotype distribution of the *Hin*dIII restriction fragments, as revealed with the whole-v-*fms* probe, in different families.

the viral sequence. Hydrolysis of this fragment by *Hind* II gave three fragments (B–D in Fig. 1). As shown in Fig. 5 (lanes 1, 2, 5, 6, 9, and 10), probing an *Eco*RI digest with fragments B, C, or D gave the following results: probe B hybridized to fragments d and e; probe C, to fragments a–d; and probe D, to fragments a and b. These results, in agreement with previous studies on the organization of the

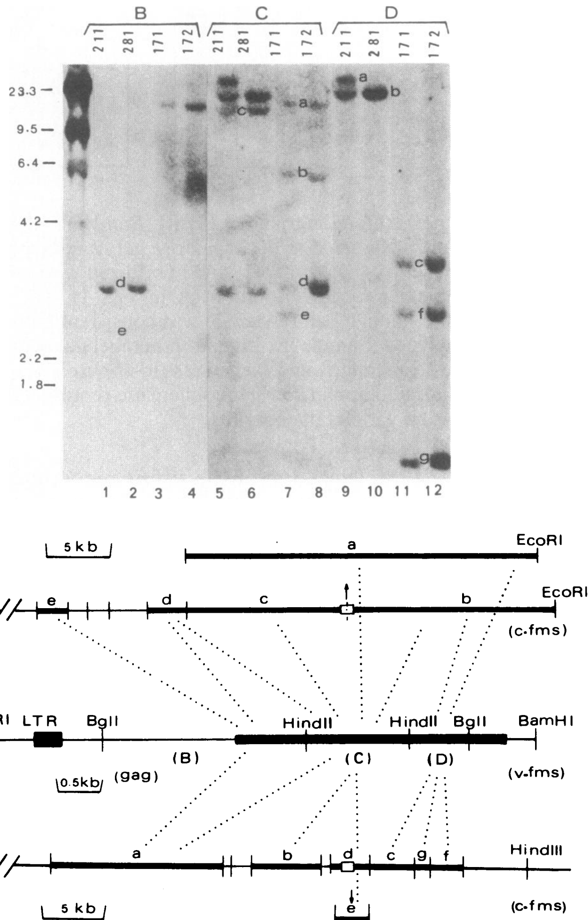


FIG. 5. (*Upper*) Four DNA samples with different phenotypes (ab, 211 and 171; bb, 281 and 172) were hydrolyzed with *Eco*RI (lanes 1, 2, 5, 6, 9, and 10) or *Hin*dIII (lanes 3, 4, 7, 8, 11, and 12) and electrophoresed in 0.8% agarose gels. After transfer, blots were hybridized with probes B, C, and D, corresponding to different parts of the viral genome (see Fig. 1). Sample identification numbers are at the tops of the lanes. Size markers are in the lane at left; values given are in kb. (*Lower*) Restriction map derived from results obtained by probing two *Eco*RI-digested DNA samples and two *Hin*dIII-digested DNA samples with different v-*fms* fragments.

c-*fms* gene (12, 13), indicate that band a might result from a mutation in the *Eco*RI site localized between fragments c and b.

In a similar way, hybridization of radiolabeled viral probes B–D to a *Hin*dIII digest gave the following results: probe B revealed fragment a only; probe C revealed fragments a, b, d, and e; and probe D revealed fragments c, g, and f (see Fig. 5, lanes 3, 4, 7, 8, 11, and 12). When these results are compared with previously published studies (12, 13), the order a, b, (d, e), c, g, f, can be proposed for the organization of these fragments in c-*fms*. The fragments d and e responsible for the *Hin*dIII RFLP were shown to lie in the proximity of the 3′ half of the c-*fms* gene.

DISCUSSION

Hybridization of *Eco*RI-digested DNA blots from 48 adults and 38 children with a viral *fms* probe reveals the existence of an RFLP for this gene. This conclusion from experimental results is easily understood by assuming the existence of two alleles, *a* and *b*: allele *b* is the most frequent one and consists of four fragments, b–e. Allele *a* is characterized by the fusion of fragments b and c, giving rise to fragment a. An examination of the unrelated adult population included in the test reveals the following distribution: 75% homozygotes *bb*; 23% heterozygotes *ab*; 2% homozygotes *aa*. Furthermore, the alleles *a* and *b* are found to be in Hardy–Weinberg equilibrium, with allelic frequencies of 13.5% and 86.5%, respectively.

Analyses of the same DNA samples hydrolyzed by *Hin*dIII have also revealed an RFLP. Here again three patterns resulting from two alleles were observed. Allele *a* is characterized by a 2.8-kb fragment (pattern A), whereas allele *b* gives a 3.2-kb fragment (pattern B). The same interpretation holds for the results obtained with *Bam*HI. Allele *a* is characterized by band d of 3 kb, and allele *b* by band c of 3.5 kb. However, the correlation detected between the three RFLPs is significant. As shown in Table 1, there is for any individual a strong correlation of the *Eco*RI, *Hin*dIII, and *Bam*HI RFLPs. Any individual found homozygous for one character is found to be homozygous for the other two. According to the identical location within the c-*fms* gene of the *Eco*RI and *Hin*dIII polymorphic sequences and the strict relationship in the segregation pattern of the *Eco*RI, *Hin*dIII, and *Bam*HI alleles *a* and *b*, one may suggest that a unique small deletion characteristic of allele *a* is responsible for the three observed RFLPs. This deletion of about 500 base pairs, as deduced from the electrophoretic mobility shift, reduces the size of the *Hin*dIII d and *Bam*HI c fragments and eliminates the *Eco*RI site located between fragment b and c (Fig. 5).

Table 1. Correlation of *Eco*RI, *Hin*dIII, and *Bam*HI RFLPs

	Phenotype			Genotype		
Sample	*Eco*RI	*Hin*dIII	*Bam*HI	*Eco*RI	*Hin*dIII	*Bam*HI
401	C	C		*ab*	*ab*	
402	A	A	A	*aa*	*aa*	*aa*
041	C	C		*ab*	*ab*	
042	B	B		*bb*	*bb*	
271	B	B		*bb*	*bb*	
272	B	B		*bb*	*bb*	
231	B	B		*bb*	*bb*	
232	B	B		*bb*	*bb*	
031	B	B		*bb*	*bb*	
032	C	C	C	*ab*	*ab*	*ab*
171	C	C		*ab*	*ab*	
172	B	B		*bb*	*bb*	
441	B	B		*bb*	*bb*	
442	B	B		*bb*	*bb*	
541	C	C		*ab*	*ab*	
542	B	B		*bb*	*bb*	
051	B	B		*bb*	*bb*	
052	B	B		*bb*	*bb*	
361	B	B		*bb*	*bb*	
362	B	B		*bb*	*bb*	
091	C	C		*ab*	*ab*	
092	B	B	B	*bb*	*bb*	*bb*
531	C	C	C	*ab*	*ab*	*ab*
532	B	B	B	*bb*	*bb*	*bb*
111	B	B	B	*bb*		*bb*
112	B		B	*bb*		*bb*
281	B		B	*bb*		*bb*
217	C		C	*ab*		*ab*
218	A		A	*aa*		

Genotype compositions of individuals tested for more than one RFLP are compared. Genotypes for all analyzed RFLPs are identical for any individual. All DNA samples summarized here were from unrelated people.

This study has been performed on a population of 48 adults and a number of their children who belong to a group of individuals already analyzed for many other genetic markers. Unfortunately, the c-*fms* gene is localized on chromosome 5 (13), about which little genetic information is available. This circumstance precludes, for the time being, the establishment of any linkage with other polymorphisms.

No chromosomal rearrangement specifically affecting chromosomes 5 at position q34, where the c-*fms* gene is located, has been reported so far. However, knowledge of the normal restriction pattern should help in the recognition of such alteration. Ultimately, since there is some reason to suspect a genetic involvement in individual predisposition to certain tumor types (21), it will be of obvious interest to compare the allelic distribution in people with malignant disorders. As shown in Figs. 3 and 4, 74% of children fromthe couples ($ab \times bb$) are heterozygotic (20 out of 27). When compared to the 50% expected frequency, this statistically valid deviation might represent some selective advantage for heterozygotes (Table 2). Because of the presence of a short deletion within allele *a*, pairing of alleles *a* and *b* during meiosis would leave an unpaired region of 0.5 kb near the distal end of the long arm of chromosome 5. This unpaired region might be recognized as such and eliminated by a conversion-like phenomenon. Alternatively, it might represent a fragile region during terminalization of the chiasma, favoring either the conversion process or the loss (with probably lethal consequence) of the distal part of chromosome 5. In both cases, the proportion of gametes with allele *a* would be increased, resulting in an increased proportion of heterozygotes.

Table 2. Segregation analysis of alleles *a* and *b* in c-*fms* locus

Parent genotypes	No. of families	No. of progeny *aa*	*ab*	*bb*	χ^2	df*	*P*
$ab \times ab$	1	1	4	0	—	—	—
$ab \times aa$	1	1	1	0	—	—	—
$ab \times bb$	7	0	20	7	6.25	1	<0.05
$bb \times bb$	2	0	0	10	—	—	—

The segregation of allele *b* in children from parents $ab \times bb$ deviates significantly from the expected distribution (50% *ab*, 50% *bb*).

*Degrees of freedom.

The analyzed samples correspond to individuals whose parents or grandparents were born in France. It would be of interest to analyze this polymorphism in populations originating in other countries.

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